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A facile preparation of highly interconnected macroporous PLGA scaffolds by liquid–liquid phase separation II

Kyu Chul Shin[a], Bong Sup Kim[a], Ji Heung Kim[a], Tae Gwan Park[b], Jae Do Nam[a], Doo Sung Lee[a,*]

[a]*Department of Polymer Science and Engineering, Sungkyunkwan University, Suwon, Kyungki 440-746, South Korea*
[b]*Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Taejon 305-701, South Korea*

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Abstract

A regular and well-interconnected macroporous (from 50 to 200 μm) poly(D,L-lactic acid-*co*-glycolic acid) (PLGA) scaffold was fabricated by means of the thermally induced phase separation (TIPS) method. Poly(L-lactic acid) (PLLA) was blended with PLGA to increase the viscosity of polymer solution; a block copolymer of poly(ethylene glycol) (PEG) with PLGA was added as a surfactant to decrease the interfacial tension between the polymer-rich and polymer-lean phases. The effect of TIPS parameters including the concentration of diblock copolymer and PLGA/PLLA ratio was also studied. The cloud-point curve shifted to higher temperatures with both increasing the PLLA composition in the PLGA/PLLA blend and the PEG contents in the additives (PEG itself and PEG–PLGA diblocks). This shifting to higher temperature increases the quenching depth during phase separation. Addition of a PEG–PLGA diblock copolymer (0.5 wt% in solution) to the PLGA/PLLA (1/1) blend polymer in a dioxane/water solution stabilized the morphology development during TIPS with respect to interconnection and macropores, and avoided segregation or sedimentation in the late stage.

Keywords: Macroporous PLGA scaffold; Thermally induced phase separation (TIPS); Liquid–liquid phase separation

1. Introduction

After the loss or failure of bodily tissues or organs, traditional surgical treatment, such as implantation of a healthy organ from a donor, is limited by the problems of immune rejection from the patient and the number of available donors [1]. The use of cell transplantation (‘tissue engineering’) is under investigation as a strategy for tissue repair and organ replacement [2–6]. Transplanted cells, cultured from a patient’s healthy tissues, can be implanted back without antagonizing the immunoisolation system. In culturing the cells, the shape of the scaffold, a temporary substrate to allow growth and specialization of the cell culture, plays an important role [7–10]. Biodegradable and biocompatible synthetic polymers, such as poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and poly(D,L-lactic acid-*co*-glycolic acid) (PLGA), have been widely utilized as three-dimensional scaffolds [11–13]. Polymeric scaffolds must be porous enough to allow a high density of cells to be seeded, yet also possess sufficient mechanical stability and a well-defined network of interconnected pores to permit ingrowth into the implanted structure [9,14]. The optimum pore size of the scaffold required differs depending on the cells or tissues; for example, pore sizes close to 20 μm are required for the ingrowth of fibroblasts and hepatocytes [15], from 50 to 150 μm for skin regeneration [16], and in the range of 100–150 μm for bone regeneration [17,18].

Numerous techniques have been developed for fabricating polyester scaffolds, including porogen leaching/salt leaching, emulsion freeze-drying, gas expansion, fiber bonding, and phase separation [19–23]. Recently, the method of freeze-drying through thermally induced (liquid–liquid) phase separation (TIPS) was developed for the preparation of biodegradable polyester scaffolds [21,23–28]. TIPS and freeze-drying were used to prepare a three-dimensional macroporous poly(L-lactic acid) (PLLA)

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* Corresponding author. Tel.: +82 31 290 7280; fax: +82 31 292 8790.
E-mail address: dslee@skku.edu (D.S. Lee).

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scaffold [23–30]. The TIPS technique provides a scaffold with a uniform pore size and high degree of interconnection, various morphologies, and good mechanical properties. The morphology can be controlled by several experimental parameters such as the quenching temperature, quenching rate, quenching period or aging time, polymer concentration, solvent to non-solvent ratio, molecular structure, and added surfactant or porogens [29–33].

Our group has reported the fabricating techniques based on liquid–liquid TIPS of polyester ternary systems to prepare macroporous PLA or PLGA scaffolds with highly interconnected structures and pore sizes ranging from 50 to 300 μm, suitable for certain cell cultures. Previously we prepared highly interconnected macroporous PLLA scaffolds with pore sizes of 50–150 μm by controlling the crystallization of PLLA during the coarsening process [29]. In other work, we found that the pore size of PLLA scaffolds with interconnected structures could be increased to greater than 200 μm through adding certain ionic compounds, surfactants, and triblock Pluronics [30,31,34].

In a preceding paper, we reported on the fabrication of a PLGA scaffold with pore sizes greater than 50 μm from PLGA/dioxane/water ternary systems. However, larger pore sizes and good interconnections between the pores were hard to get and the morphology development during quenching process was not stable because of the low viscosity of the PLGA solution [32].

In this study, the effect of blend with PLLA and the adding a PEG–PLGA diblock copolymer was investigated in order to get regular and highly interconnected macroporous PLGA/PLLA scaffolds with pore sizes greater than 200 μm. The scaffold morphology was also investigated by adjusting some of TIPS parameters.

2. Experimental

2.1. Materials

PLGA (High IV 50/50 lactic acid/glycolic acid (LA/GA); number-average molecular weight M_n 1.3×10^5; inherent viscosity $\sim$0.73 dL g^{-1}) was purchased from Alkermes. PLLA (Lacty 5000; M_n 2.18×10^5; PDI 1.55) was purchased from Shimadzu. 1,4-Dioxane and deionized water were a good solvent and non-solvent for PLGA/PLLA. Monomethoxy poly(ethylene oxide) (PEO; M_n 5000, PDI 1.1) was purchased from Aldrich and purified before use by dissolution in dry chloroform then precipitation from *n*-hexane. L-Lactide (Boehringer Ingelheim) was purified before use by recrystallization from thoroughly dried ethyl acetate under a dry nitrogen atmosphere and sublimation. PEG–PLGA diblocks (Table 1) were synthesized and characterized as reported previously [35].

Table 1
Characterization of the synthesized diblock copolymers

	Copolymer	Block M_n/g mol^{-1a}
Diblock1	PEG–PLGA	5000–2848
Diblock2	PEG–PLGA	5000–4532

[a] Number-average molecular weight calculated from ^{1}H NMR measurements.

2.2. Phase diagram

The cloud-point curves of the PLGA/PLLA blend in the presence of PEG or PEG–PLGA diblocks were determined by visual turbidimetry. PLGA/PLLA (3, 6, 9, and 12 wt%) and PEG or PEG–PLGA diblocks (0.2, 0.5, or 1 wt% in whole solution) were added to a 4 mL vial tube, equipped with a magnetic stirrer and 1,4-dioxane/water mixture (87/13 wt/wt) as solvent, and then dissolved at 58 °C for 3 h. The homogenous PLGA/PLLA solution was reheated to about 10 °C above the expected cloud-point temperature, then slowly cooled in steps of 1 °C, allowing the system to equilibrate for 10 min at each new temperature. The cloud-point was reported at the temperature at which the clear solution became visually turbid. The gelation point was determined by inverting the vial horizontally after it had been maintained for 10 min at a constant temperature, as described previously [29].

2.3. Preparation of PLGA/PLLA scaffolds

PLGA/PLLA (9 wt%) solutions in 1,4-dioxane/water (87/13 wt/wt) containing PEG or PEG–PLGA diblocks (0.2, 0.5, or 1 wt%) were prepared. The sample was reheated to 15 °C above the measured cloud-point temperature, then placed into a water bath preheated to the quenching temperature. The sample remained for 2, 10, 30, 60, or 120 min at this temperature. The annealed sample was directly immersed in liquid nitrogen for 1 h, and then one small hole was cut in the cap of vial to allow the solvents to depart. The freeze-drying was performed at −77 °C and 7 mTorr for 3 days in order to remove solvents and thereby obtain the macroporous scaffolds.

2.4. Morphology characterization

The macroporous morphology of the scaffolds was observed using scanning electronic microscopy (SEM, Hitachi S-2400). Fracture-frozen cross-sections of the scaffold were mounted on an Al stub covered with a carbon adhesive and then coated with Pt particles.

The size of pores PLGA/PLLA scaffold and PLGA scaffold (of the previous paper) were measured from micrographs to compare the size development during the fabrication. The average value from at least five pores was calculated from magnified micrographs.

3. Results and discussion

PLLA is semi-crystalline polymer, with partial crystallization occurring during scaffold fabrication, lending it rather strong mechanical properties. PLGA/PLLA is a miscible blend in the bulk state as well as in solution. This PLGA/PLLA blend is soluble in dioxane/water (87/13), as are the PLLA and PLGA solutions we have previously used to prepare scaffolds [29–32]. In general PLGA with 75/25 LA/GA was usually used for soft tissue regeneration; PLGA with a 50/50 LA/GA ratio has poor mechanical properties and has not been used as a scaffold. We tried to fabricate scaffolds using a PLGA/PLLA(1/1 wt ratio) blend and keeping LA/GA(74/26) total composition, although this blend may have a different degradation rate to a pure PLGA (LA75/GA25) copolymer.

3.1. Phase diagram

The sharp change in turbidity of the (PLGA/PLLA)/-dioxane/water ternary system at the cloud-point temperature is the result of liquid–liquid demixing. Fig. 1 shows the cloud-point curves for systems of pure PLGA/PLLA and with added PEG. The cloud-point curve shifted to higher temperatures with increasing polymer concentration. The cloud-point curve also shifted with increasing PLLA concentration because PLLA is more hydrophobic than PLGA: the GA composition in PLGA/PLLA 9/1, 7/3, and 1/1 blends are 45, 35, and 25%, respectively. When hydrophilic PEG was added to this solution, the curve shifted to still higher temperatures due to changing the hydrophilicity of solvent. We selected 0.5 wt% of additives such as PEG, diblock1, and diblock2 for further study.

The cloud-point curves in the presence of various additives shifted to higher temperatures in the order of PEG > diblock1 > diblock2, as shown in Fig. 2. It is known that phase separation is assisted by adding diblock or triblock copolymer as a surfactant, because of the amphiphilic effect of the added surfactant serving as a nucleus for phase separation; this effect can be related to the additive's molecular weight, hydrophobic/hydrophilic block ratio, and block lengths [31,34]. Diblock2 (Table 1) has similar hydrophilic (PEG) and hydrophobic (PLGA) block lengths, in contrast with the smaller PLGA block length of diblock1. The hydrophilicity of additives is thus in the order PEG > diblock1 > diblock2, and increasingly hydrophilic additives raised the cloud-point to higher temperatures. An increasing PEG content in additives enhanced the interaction with water, and caused liquid–liquid demixing to shift to higher temperatures.

Because of the partial crystallization of PLLA, the PLLA component of the PLGA/PLLA solution showed gelation when the polymer concentration exceeded the sedimentation boundary (> 8 wt%, line A in Fig. 2) and the solution was cooled to below the gelation-point temperature [29]. The gelation-point temperature gradually increased with increased polymer concentration. Below the sedimentation boundary, the polymer solution separated into two layers, a polymer-rich and polymer-lean phase. For the PLGA solution, gelation was not observed even on cooling to 0 °C due to its amorphous nature. In addition, at long times during the coarsening process the mixture separated into two layers as a result of sedimentation under gravity, thereby separating the coarsened phases on the basis of their density. In contrast, the PLLA solution shows a sedimentation boundary at around 4.5 wt% concentration. That the sedimentation boundary of PLGA/PLLA is located at

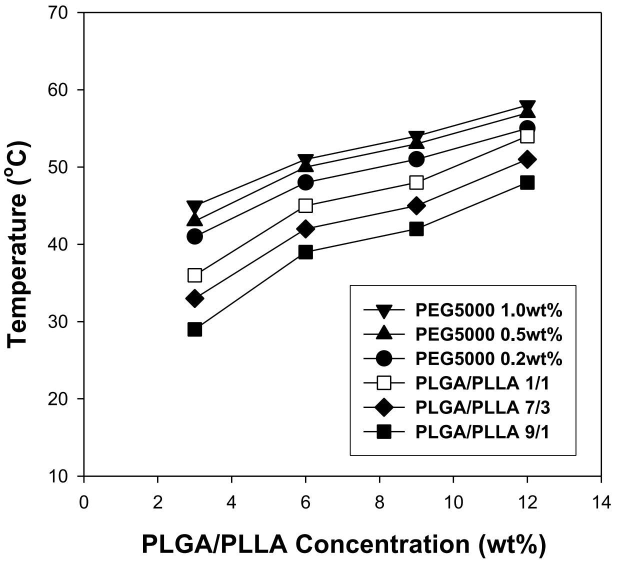

Fig. 1. Cloud-point curves of PLGA/PLLA solutions and of PLGA/PLLA (1/1) with added PEG (M_n 5000).

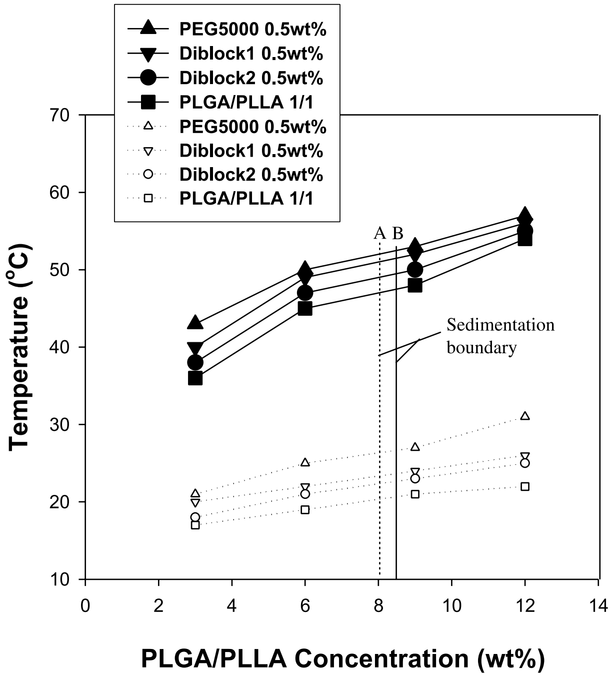

Fig. 2. The effect of additives on the cloud- and the gelation-point curves.

around 8 wt% concentration is quite understandable from the crystalline and amorphous nature of the polymer.

Fig. 2 shows that the gelation-point curves of the ternary system are raised by addition of PEG or diblocks, for the same reason as the cloud-point increase. The sedimentation boundary (>8.5 wt%, line B in Fig. 2) shifted to a slightly higher polymer concentration with increased PEG content.

3.2. *Effect of PLGA/PLLA composition and quenching temperature*

As mentioned in Section 1, the final porous morphology (pore size, pore shape, porosity) of a scaffold is determined by the thermodynamic state of the solution at the time of quenching prior to freeze-drying. The thermodynamic state of the solution depends on the parameters such as the quenching temperature, polymer concentration, solvent composition, and aging time. In our preceding paper [29], optimum processing conditions for preparing a regular PLLA scaffold of pore size 50–150 μm were polymer concentration (4.5 wt%) and solvent (dioxane/water, 87/13 wt/wt). On the basis of this formulation, PLLA scaffolds with pore sizes of up to 300 μm were easily prepared upon including additives [31].

The effect of PLGA/PLLA composition on the scaffold morphology was tested at 3 °C after aging (10–60 min; Fig. 3). In this case, very low temperature (3 °C) was used because PLGA/PLLA blend ratios of 7/3 and 9/1 were difficult to get porous structure due to low viscosity at high temperature. As shown Fig. 3(a), 1/1 PLGA/PLLA (9 wt%) formed a scaffold of regular and some open-pored morphology. The pore interconnection was better, pore size bigger, and structure better developed compared to those from the PLLA-poorer systems. The added PLLA increased both the interaction between the polymers and the viscosity of the solution, so that the porous structure possesses a greater mechanical strength.

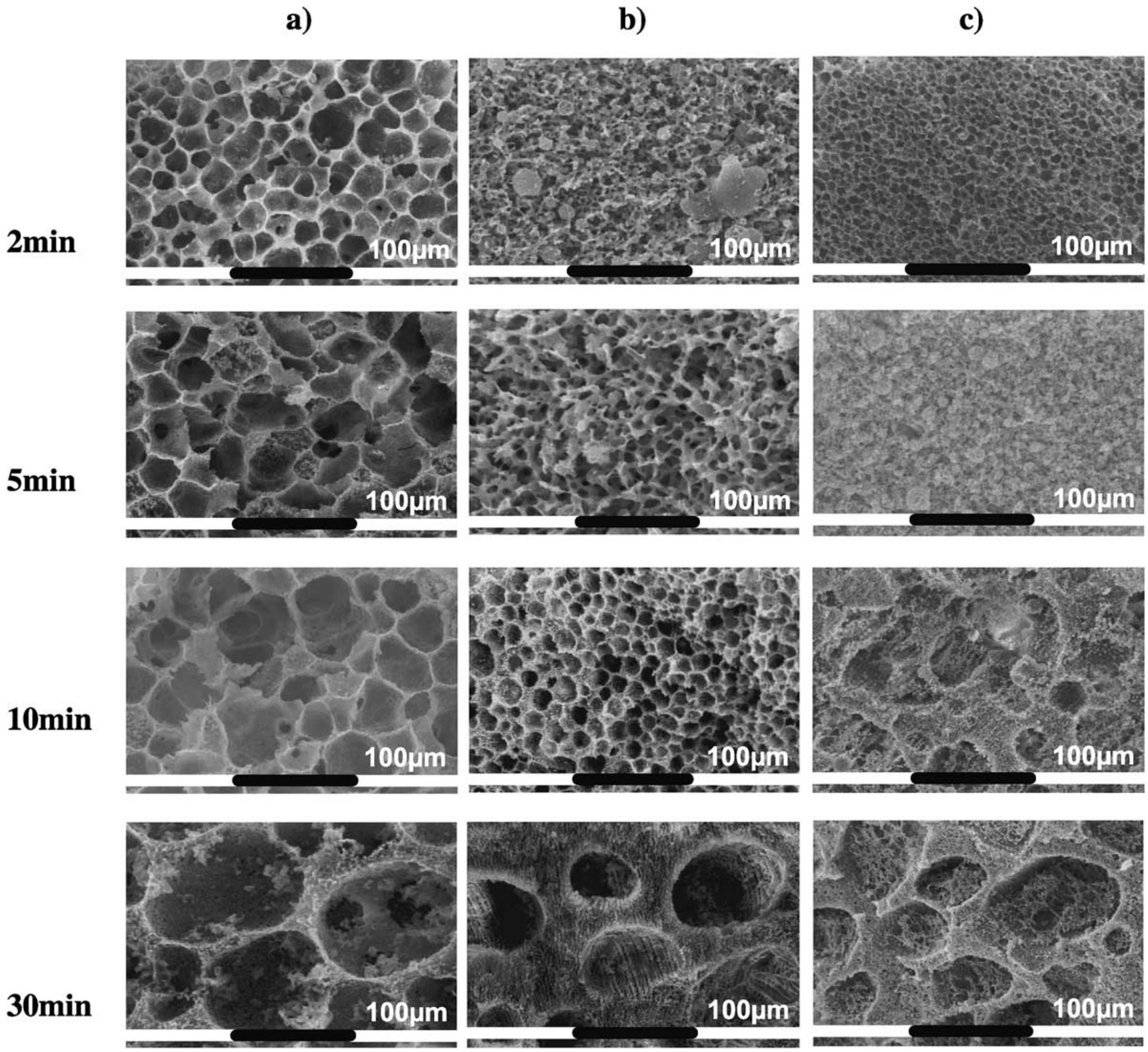

Fig. 3. SEM images of the scaffold prepared from PLGA/PLLA solutions (9 wt%, in dioxane/water 87/13) as a function of aging time (2–30 min) at a quenching temperature of 3 °C. PLGA/PLLA blend ratios are (a) 1/1, (b) 7/3, and (c) 9/1.

From these results, we proceeded to fabricate scaffolds using a 1/1 PLGA/PLLA blend.

Fig. 4 shows the morphology of scaffolds prepared from 1/1 PLGA/PLLA (9 wt%) at three quenching temperatures, 20, 30, and 40 °C. The pore size increased continuously with aging time to 60 min. Although the pores were closed and not well interconnected, the scaffold pore size was greater than 100 μm after 60 min at a quenching temperature of 20 °C. For quenching temperatures of 30 and 40 °C, the morphology developed slowly after 10 min because of the low thermodynamic driving force.

3.3. *Effect of additives*

At the early stages the scaffold morphology is determined by the initial thermodynamic driving force, which in turn is dependent upon the quenching depth. Cloud-point temperatures are raised by addition of diblocks or PEG as shown Fig. 2, which make a deeper quenching depth at a given quenching temperature. Fig. 5 shows the effect of additives (PEG, diblock1, diblock2; 0.5 wt%) on the PLGA/PLLA scaffold morphology at a quenching temperature of 30 °C. The phase diagram in Fig. 2 shows that three

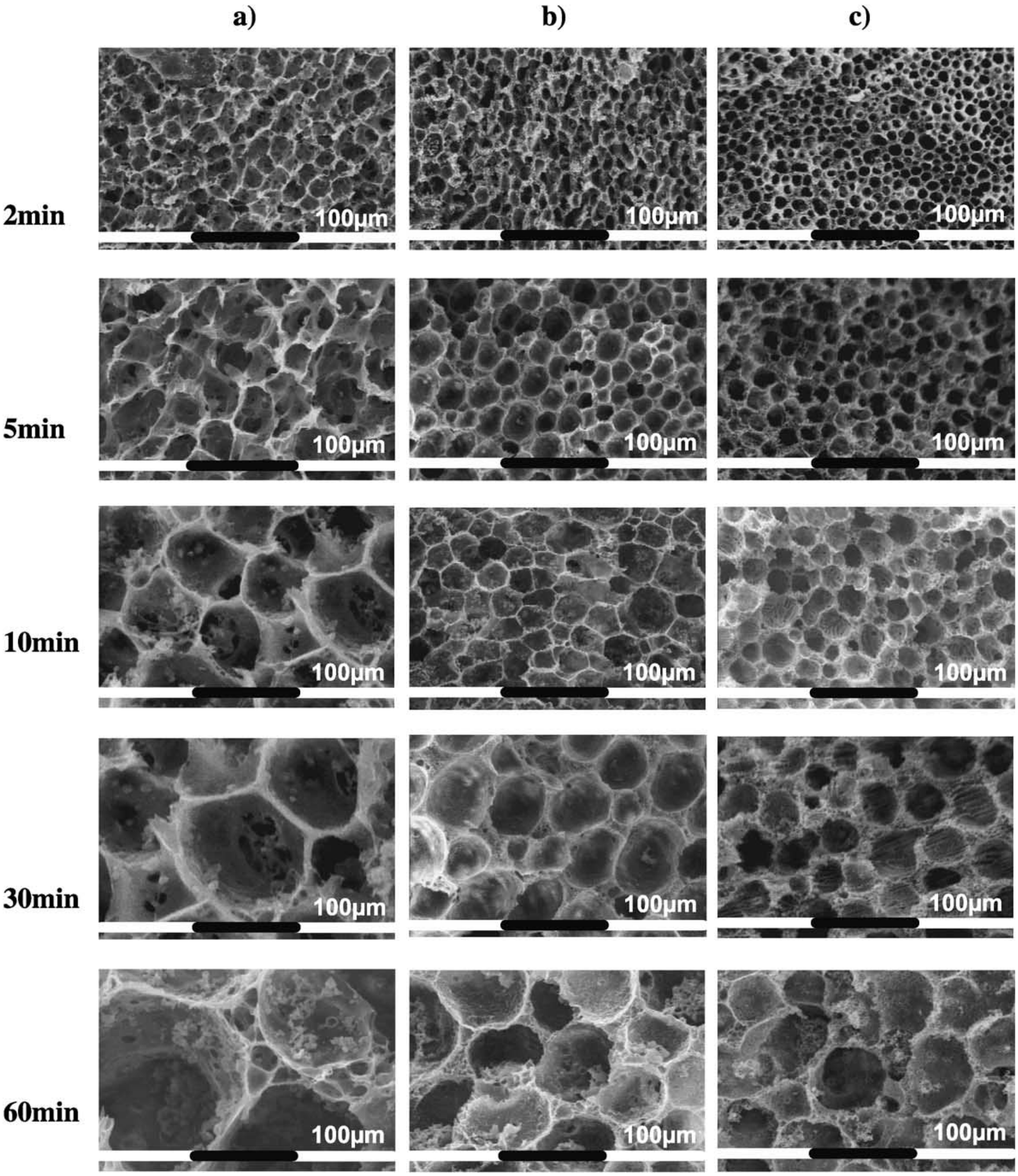

Fig. 4. SEM images of the scaffold prepared from PLGA/PLLA solution (1/1, 9 wt%, in dioxane/water 87/13) as a function of aging time (2–60 min) at quenching temperatures of (a) 20 °C, (b) 30 °C, and (c) 40 °C.

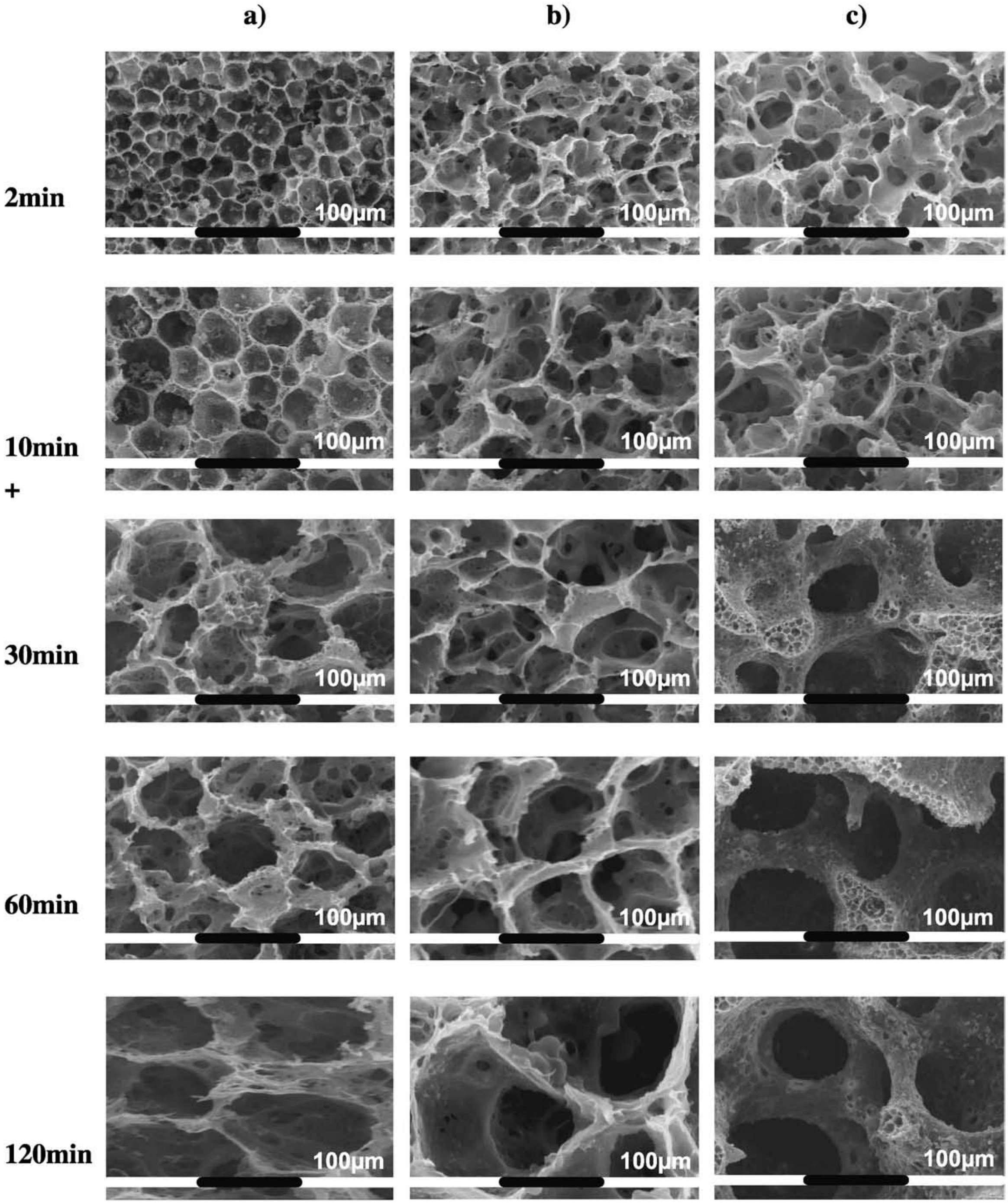

Fig. 5. SEM images of the scaffold prepared from PLGA/PLLA solution (1/1, 9 wt%, in dioxane/water 87/13) with additives (0.5 wt%) as a function of aging time (2–120 min) at a quenching temperature of 30 °C. Additives (a) PEG, (b) diblock1, and (c) diblock2.

quenching temperatures (20, 30, 40 °C) are located in the unstable (spinodal) region. The interconnected and open porous structure was observed at an early stage (< 10 min). The pore size of the scaffold increased with aging, reaching sizes greater than 150 μm after 60 min. The walls between pores were not destroyed even after extended aging (120 min), as shown in Fig. 5(b) and (c), in contrast to the PLGA and PLGA/PLLA systems without additives. When the pure PLGA/PLLA solution was quenched at 30 °C, the pore sizes and their changes with time were smaller, even though the system had a higher driving force. Because the system was located at lower temperature than its gelation-point temperature, the crystallization of PLLA prevented phase separation and restricted decreases in pore sizes at later stages (> 30 min).

Addition of diblock1 or diblock2 raised the thermodynamic driving force in comparison to the pure PLGA/PLLA solution. The interconnected pore sizes of the scaffold prepared without diblock copolymers were, in the early stages (<2 min), not smaller than those of the scaffolds prepared with diblock copolymers. However, after 10 min the pore size of the PLGA/PLLA scaffolds gradually increased, in contrast with the scaffold without diblock copolymers, and after 120 min a uniform macroporous structure were observed, a result arising from the growth of larger structures at the expense of smaller ones [37], as shown in Fig. 5(c). The addition of diblocks to the ternary system stabilized the pore structure during phase separation and prevented sedimentation. This is distinctly observed for the systems containing diblocks, as presented in Fig. 5(b) and (c). For these systems, the regular, open, and well-interconnected macropores in the size range 150–200 μm were fabricated after 120 min without coarsening. Moreover, the wall structures appeared more compact and mechanically stronger.

These diblocks cause a lowering of the interfacial tension, which is important at the later stages of phase separation. At this stage, the phase-separation kinetics were mainly controlled by the motion of interfaces driven by interfacial tension [36]. Lowering the surface tension decelerates phase separation, which allows greater time for stabilization of the interconnections. The hydrophilic/hydrophobic ratio of the block copolymer played a role in decreasing the interfacial tension. Diblock2, with a low hydrophilic/hydrophobic ratio, was more effective in obtaining larger pore size and good interconnections in the scaffold than diblock1. This indicates that a suitable PEG/PLGA ratio and appropriate molecular weight decrease interfacial tension more effectively, and hence give better stabilization of the interconnections between macropores. The diblock-added systems had no tendency towards segregation and sedimentation.

The PEG-added system has the largest quenching depth (18 °C) than any other system at 30 °C, a depth located at 1 °C above its gelation-point temperature. After a short aging time (<2 min), the higher thermodynamic driving force produced larger pore sizes. However, after 10 min, the pore size growth gradually decelerated during the phase-separation process due to a higher viscosity around the gelation point, as shown in Fig. 5(a). There was a similar effect with added PEG but it was not significant compared to the diblocks.

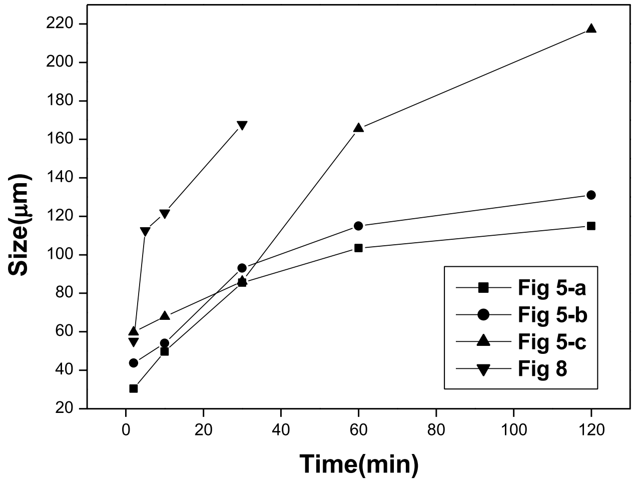

Fig. 6. The pore size development during fabrication for the above Fig. 5(a)–(c) and PLGA scaffold at −7 °C of Fig. 8 in the previous paper [32].

The pore size development during fabrication is shown in Fig. 6. The PLGA scaffold fabricated at −7 °C has best morphology prepared in the previous paper [32], but the development rate was very fast (within 30 min) and the coalescence morphology appeared after 30 min. Moreover, its morphology was somewhat closed cell structure. While the structure of PLGA/PLLA scaffold with diblock copolymer shows the rather slow development up to 120 min. Their morphology was open cell structure and the size of pores could be precisely controlled by aging time.

4. Conclusions

Blending PLLA with PLGA could provide a new method for preparing macroporous scaffolds of soft PLGA by the TIPS method. Added PLLA increased the viscosity of the PLGA/PLLA solution, to make mechanically stronger porous scaffolds, and raised the cloud-point curve, to create larger thermodynamic driving forces. The addition of amphiphilic diblocks decreased the interfacial tension and enabled the formation of well-interconnected, mechanically strong macroporous scaffolds. The pore size of the PLGA/PLLA scaffold ranged from 50 to 200 μm and their morphology was controlled by the processing parameters (quenching temperature, aging time, polymer concentration, and composition and length of diblocks).

The combination of these two methods—blending with a crystalline polymer and adding diblocks as a surfactant—provides a new method to prepare open, regular, and well-interconnected macroporous scaffolds of PLGA and other soft materials using the TIPS method.

Acknowledgements

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